


U.S.C. §132. Accordingly, entry of the amendments prior to examination of the application is respectfully requested.

Respectfully submitted,

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BOX PATENT

Attorney Docket No. 24512-X

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of

Arnold J. REUSER et al.

Serial No.: 09/886,477

Filing Date: June 22, 2001

For: **METHODS OF PURIFYING HUMAN ACID ALPHA-GLUCOSIDASE**

Appendix A

Please cancel presently pending claims 38-39 and amend the following claims as indicated in the following marked up copy of the claims.

3. (Once Amended) The method of claim 2 [or claim 3], wherein the anion exchange column is Q-Sepharose.

4. (Once Amended) The method of claim [4] 3, wherein the sample is applied to the Q-Sepharose column in low salt buffer and is eluted from the column in an elution buffer of higher salt concentration.

5. (Once Amended) The method of claim 2 [or claim 3], wherein the anion exchange column is copper chelating Sepharose.

7. (Once Amended) The method of claim 2 [or claim 3], wherein the hydrophobic interaction column is phenyl Sepharose.

8. (Once Amended) The method of claim 2 [or claim 3], wherein the hydrophobic interaction column is Source Phenyl 15.

10. (Once Amended) The method of [any one of claims] claim 2 [to 9], further comprising repeating steps (a) and (b) and/or

(c) until the a-glucosidase has been purified to 95%, preferably 99%, more preferably 99.9% w/w pure.

11. (Once Amended) The method of [any one of claims] claim 2 [to 10], wherein the sample is milk produced by a transgenic mammal expressing the a-glucosidase in its milk.

14. (Once Amended) The method of [any one of claims] claim 11 [to 13], further comprising centrifuging the milk and removing fat leaving skimmed milk.

18. (Once Amended) The method of [any preceding] claim 1, wherein the sample has a volume of at least 100 liters.

22. (Once Amended) At least 95%, preferably at least 99%, more preferably at least 99.9% w/w pure human [Human] acid a-glucosidase [of any one of claims 19-21] produced by the process of [any one of claims 1-18] claim 1.

24. (Once Amended) A pharmaceutical composition comprising human acid a-glucosidase as claimed in [any one of claims 19-21] claim 19.

25. (Once Amended) Human acid a glucosidase of [any one of claims 19-21] claim 19 for use as a pharmaceutical.

27. (Once Amended) The use of human acid a-glucosidase of [any one of claims 19-21] claim 19 for the manufacture of a medicament for treatment of human acid a-glucosidase deficiency.

28. (Once Amended) The use of human acid a-glucosidase of [any one of claims 19-21] claim 19 for the manufacture of a medicament for intravenous administration for the treatment of human acid a-glucosidase deficiency.

32. (Once Amended) A method as claimed in [any of claims]

claim 29 [to 31] being a batch procedure.

33. (Once Amended) A method as claimed in [any of claims] claim 29 [to 31], wherein the hydroxylapatite is in the form of a column, optionally the method is a liquid column chromatography procedure.

34. (Once Amended) A method as claimed in [any of claims] claim 29 [to 33], wherein the heterologous protein [ie] is selected from the group consisting of [lactoferrin, transferrin, lactalbumin, factor IX, growth hormone, a-anti-trypsin,] lactoferrin, transferrin, lactalbumin, coagulation factors such as factor VIII and factor IX, growth hormone, a-anti-trypsin, plasma proteins such as serum albumin, C1-esterase inhibitor and fibrinogen, collagen, immunoglobulins, tissue plasminogen activator, interferons, interleukins, peptide hormones, and lysosomal proteins such as a-glucosidase, a-L-iduronidase, iduronate-sulfate sulfatase, hexosaminidase A and B, ganglioside activator protein, arylsulfatase A and B, iduronate sulfatase, heparan N-sulfatase, galactoceramidase, a-galactosylceramidase A, sphingomyelinase, a-fucosidase, a-mannosidase, aspartylglycosamine amide hydrolase, acid lipase, N-acetyl-a-D-glycosamine-6-sulphate sulfatase, a-and ss-galactosidase, ss-glucuronidase, ss-mannosidase, ceramidase, galactocerebrosidase, a-N-acetylgalactosaminidase, and protective protein and others including allelic, cognate or induced variants as well as polypeptide fragments of the same.

35. (Once Amended) A method as claimed in [any of claims] claim 29 [to 24], wherein the heterologous protein is not one

[illegible]

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